

AMENDMENTS TO THE SPECIFICATION

At paragraph [0012] replace the paragraph with the following:

[0012] A method for initiating primary cultures of corneal endothelial cells, including that of human origin, by using dissection enables the culture to start from a low initial density (100-500 cells/mm²) and expand from a seeding density of 1 to 32. These cells can be effectively passaged 7 to 8 times without losing their morphological integrity and physiological functions, such as formation of tight intracellular junctions and Na/K pump activation. The corneal endothelial cultures can be maintained in a commonly used fetal bovine serum (FBS) supplemented culture medium enriched with selected growth factors such as fibroblast growth factors 1 and 2 (FGF1, FGF2), epidermal growth factors (EGF), transforming growth factor β (TGF β), endothelial cell growth factor (ECGF), and other growth factors known in the art of cell culture. In particular, if the corneal endothelial cells are propagated in a natural extracellular matrix as supplied by bovine corneal endothelial culture, or a synthetic attachment protein mixture containing such components as fibronectin, laminin, collagen type I and IV, and RGDS (SEQ ID NO: 1), or on a carbon plasma deposit known as diamond-like carbon (DLC), the culture will assume a more hexagonal morphology upon repeated passage at high split ratio (1:32 or 1:64) for up to 10 passages. The generation of a large pool of corneal endothelial cells, especially that of human origin, can be banked in cryo-storage and used for future cell transplantation procedures.

At paragraph [0024] replace the paragraph with the following:

[0024] Prior to seeding the cells onto the denuded corneal surface, a predetermined mixture of attachment proteins containing fibronectin (ranging from 0.1 μ g to 500 μ g/ml in PBS), laminin (0.1 μ g to 500 μ g/ml in PBS), RGDS (SEQ ID NO: 1) (0.01 μ g to 100 μ g/ml in PBS), collagen type IV (ranging from 0.1 μ g to 1000 μ g in 0.1 M acetic acid) will be added to the denuded surface (Descemet's membrane) and incubated at 4° C. for a period ranging from 5 to 60 minutes. The residual protein mixture will be removed after the incubation period, and the cornea is rinsed three times with PBS and placed endothelial side up on a Teflon concave holder. An 11 mm

diameter button will be punched out with a size 11 trephine. This button will be ready to receive the cultured corneal endothelial cells.

At paragraph [0026] replace the paragraph with the following:

[0026] Alternatively, the process of maintaining human corneal endothelial cells in culture, expansion of the corneal endothelial cells, and the preparation of the attachment protein can be used to coat artificial cornea stroma generated from polymer-gel composition. Briefly, a poly-gel stroma can be molded into a cornea shape, and the concave side (endothelial side) will be treated with a mixture of attachment proteins and growth factors such as fibronectin (ranging from 0.1 to 500 $\mu\text{g/ml}$ in PBS), laminin (ranging from 0.1 to 500 $\mu\text{g/ml}$ in PBS), RGDS (SEQ ID NO: 1) (ranging from 0.01 to 100 $\mu\text{g/ml}$ in PBS), collagen type IV (ranging from 0.1 μg to 1000 μg in 0.1 M acetic acid), FGF (10 to 400 ng/ml in PBS), EGF (10 to 400 ng/ml in PBS), or TGF β (1 to 100 ng/ml in PBS). After incubation at 4° C. for a period ranging from 10 minutes to 2 hours, the artificial stroma will be rinsed three times with PBS, and cultured human corneal endothelial cells at a density of about 50,000 to about 10^6 cells/ml preferably about 150,000 to 250,000 cells/200 ml of culture medium (DMA-H16 with 5% FCS or a mixture of attachment proteins containing fibronectin, laminin, RGDS (SEQ ID NO: 1), and collagen type IV) will be added to a corneal button of 11 mm diameter. A layer of (10 mg/ml sodium hyaluronate, 0.1 to 0.5 ml) will be applied carefully onto the cell layer as a protectant, and the button will be incubated at 37° C. in a 10% CO₂ incubation for a period ranging from 10 minutes to 24 hours. The artificial corneal button will be rinsed 3 times with PBS after the incubation and will be ready for transplantation.

At paragraph [0036] replace the paragraph with the following:

[0036] After trephination, the denuded cornea button will be placed endothelial side up again in a holder. A solution of attachment proteins containing fibronectin at a concentration ranging from 10 μg to 500 $\mu\text{g/ml}$ in PBS, laminin (10 ~~82~~ μg to 500 $\mu\text{g/ml}$ in PBS), RGDS (SEQ ID NO: 1) (1 μg to 100 $\mu\text{g/ml}$ in PBS), collagen type IV (10 μg to 1000 μg in 0.1 M acetic acid), b-FGF (1 to 500 ng/ml in PBS), EGF (1 ng to

500 ng/ml in PBS) will be added carefully onto the denuded cornea button. The specimen is allowed to incubate at 4° C. for a time ranging from 5 minutes to 2 hours, at the end of which the cocktail will be removed and the cornea rinsed 3 times with PBS.

At paragraph [0039] replace the paragraph with the following:

[0039] To generate the artificial matrix, fibronectin, laminin and RGDS (SEQ ID NO: 1) will be dissolved at concentrations of about 100 ~~82~~ $\mu\text{g/ml}$ in distilled water, and collagen type IV is dissolved at a concentration of about 1 mg/ml in 0.01% acetic acid. Basic FGF is dissolved at a concentration of about 100 ~~$\mu\text{g/ml}$~~ ng/ml in bovine serum albumin (0.05% w/v). All the materials are mixed together in a 15 ml centrifuge tube and swirled gently to avoid bubbling. The mixture is then incubated at 4° C. for two hours.